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With better matching due to genomic tissue typing and improved immunosuppression, hematopoietic stem cell transplant (HSCT) using HLA-matched unrelated donors (URD) have similar outcome as using HLA-identical sibling donors (sib). Do some leukemia patients benefit from an URD transplant, because of a stronger graft-versus-leukemia (GVL) effect? We analyzed the outcome in 941 URD (8/8 HLA-allele matched) transplants, compared to 3158 sib HSCT for acute leukemia (AML and ALL) and chronic myeloid leukemia (CML) receiving myeloablative conditioning between 1995–2004 and reported to the CIBMTR. Transplant-related mortality (TRM), relapse, survival and leukemia-free-survival (LFS) were compared for each disease. In a Cox proportional hazard regression model including variables important for outcome, acute and chronic GVHD were added as time-dependent variables.

Results: Using URD vs. sib HSCT, TRM was increased in early AML (40% vs. 24%, $p = 0.001$), and advanced ALL or AML (44% vs. 31%, $p = 0.01$). Among other risk factors, TRM was associated with URD ($p < 0.001$), advanced disease and increasing donor and recipient age. Relapse was similar using URD or sib donors; AML CR1 (5-year relapse 22% vs. 15%), intermediate disease (21% vs. 22%), advanced (43% vs. 46%), ALL early (15% vs. 23%), intermediate (36% vs. 32%) and advanced (48% vs. 52%), and for CML early (5% vs. 6%), intermediate disease (16% vs. 22%) and blast crisis (39% vs. 35%) (ns). In the Cox model, relapse was decreased in patients with chronic GVHD for AML and ALL and in patients with acute GVHD for CML. LFS was decreased in AML CR1 and advanced disease, and CML in blast crisis in URD vs. sib transplants. **To Conclude:** Relapse was the same using URD compared to sib HSCT, suggesting similar GVL effect. TRM was increased and LFS was decreased using URD. Therefore, a sib donor should be the first choice.

34

CHARACTERIZATION OF B CELL TARGET ANTIGENS IN PATIENTS WITH CHRONIC GRAFT VERSUS HOST DISEASE

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Patients with chronic graft versus host disease (cGVHD) develop antibody responses against a variety of target antigens 4–8 months after hematopoietic stem cell transplantation (HSCT). These antibodies can be specifically directed against amino acid polymorphisms that distinguish recipient from donor proteins (allo-antibodies) or against normal or tumor-associated proteins that are identical in the recipient and donor (auto-antibodies). We used high-density protein microarrays (ProtoArray Protein Microarrays V4, Invitrogen) to define the spectrum of antibody targets in patients with cGVHD after HSCT. These protoarrays contain over 8,000 unique proteins individually spotted on nitrocellulose-coated slides under native conditions. We used 1-year post-HSCT plasma from 10 patients with cGVHD for antibody profiling. The reactivity of each patients cGVHD plasma was compared to a sample obtained from the same patient prior to HSCT as well as to the patients donor. All 30 arrays were from the same lot and were processed by the same operator to enhance consistency. Plasma was tested at a dilution of 1:500 and results were analyzed using ProtoArray Prospector software (InVitrogen). Overall, 98 proteins were targeted by cGVHD plasma and not by any of the pre-HSCT or donor samples. Only 4 of the 98 proteins were targeted by 2 or more cGVHD patients: protein kinase D3 (nu), ribonucleoprotein autoantigen (Ro/SSA), aldehyde dehydrogenase 7, and pyruvate dehydrogenase E1 component alpha subunit. Several proteins identified by cGVHD antibodies were surface membrane or secreted proteins. These include PDGFR-alpha, PDGFR-beta, FGFR-3, FGFR1 oncogene partner, adrenergic-beta receptor kinase, poliovirus-receptor related-3, liprin beta-2, PMEPA1/STAG1, neuropeptide-Y and serpin peptidase inhibitor. Antibodies to these targets may have direct activating or inhibitory functional effects or may initiate lysis of target cells through complement-mediated killing or antibody dependent cellular cytotoxicity. These effects can occur independently of effector T cell responses to these proteins. These results demonstrate that patients with cGVHD develop antibodies specific for a largely unique spectrum of target antigens. These target antigens include surface membrane and secreted proteins with potential functional capacities and this may explain why B cell-directed therapy can improve some clinical manifestations of cGVHD.

35

HIGH BAFF AND BCR-ACTIVATED B CELLS IN PATIENTS WITH PROGRESSIVE CHRONIC GRAFT VERSUS HOST DISEASE AFTER RITUXIMAB TREATMENT

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We recently showed that high levels of B cell Activating Factor (BAFF) after allogeneic hematopoietic stem cell transplantation (HSCT) are associated with the development of chronic graft versus host disease (cGVHD). Clinical responses observed after treatment with rituximab (anti-CD20) also suggest a role for B cells in the immune pathology of cGVHD, but the effect of rituximab on BAFF levels and B cell repertoire in patients with cGVHD is unknown. In patients with autoimmune disease treated with rituximab, BAFF levels are elevated for 1–2 months after B cell depletion. Since high BAFF promotes survival and differentiation of human B cell receptor (BCR)-activated (CD27+) B cells into Ig-secreting cells there is concern that high BAFF after rituximab may contribute to clinical relapse. We studied 20 patients who received rituximab (375 mg/m^2/week × 4 consecutive weeks for one or two cycles) for steroid-refractory cGVHD. Patients were tested a median of 25 months after starting treatment. At the time of analysis, 9 had progressive cGVHD, defined as worsening signs or symptoms in any organ system or the requirement of additional systemic therapy and 11 had stable or improved cGVHD, measured by reduction in immune suppression and overall clinical assessment. While median CD19+ B cell number did not differ prior to rituximab treatment (104.5×10^6/L versus 203.7×10^6/L, $p = 0.60$, respectively), CD19+B cell number was significantly lower in the 9 patients with worsening cGVHD at 25 months. Median BAFF levels were also significantly higher in this group. To examine whether high BAFF levels affected B cells in these patients, we examined BAFF-R expression on CD19+ B cells by flow cytometry. The median fluorescence intensity (MFI) of BAFF-R expression was lower in the progressive disease group compared to the stable/improved group (0.62 versus 1.67 MFI, respectively). Finally, patients with progressive cGVHD had increased proportions of CD27+ B cells, indicating that the pool of circulating B cells contains a higher fraction of activated cells in these patients (Table 1). Despite persistent B lymphopenia after rituximab, patients with progressive cGVHD had significantly higher BAFF levels associated with a significant increase in the proportion of CD27+ BCR-activated circulating B cells. These data provide a rationale for investigation of BAFF antagonists in the treatment of cGVHD.

BAFF levels & B cell phenotype 25 months after rituximab therapy for cGVHD

	Stable/Improved (n = 11)	Progressed (n = 9)	p-value
Total B Cell No. ($\times 10^6$)/L	124.7	3.8	0.002
BAFF (ng/ml)	2.58	7.72	0.01
% BAFF-R on B Cells	68.4	2.2	0.004
% CD27 on B Cells	9.0	70.9	0.04